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Problems of Epidemiological Evidence*

Problems associated with the use of epidemiological evidence to evaluate the carcinogenicity of metals are, in the main, those associated with epidemiological enquiry in general. There are, however, some additional difficulties that arise from the variety of ways in which exposure to metals can occur, the variety of metal species and compounds, and the frequency of mixed exposure. We shall, therefore, review some of these problems, and the ways in which they can be overcome, before reviewing the evidence relating to individual elements.

One of the greatest difficulties is to obtain a population for study that is large enough to distinguish the effects of a specific hazard from the effects of the random variation of small numbers or alternatively to be confident that no material risk exists, when negative results are obtained (the problem of statistical power and its relation to sample size). These cannot always be overcome by increasing the size of the population, as the number of people exposed to high concentrations of the agent may be small and extension of the study to include larger numbers will only dilute the results, if the additional subjects have not been intensively exposed. In studies of cancer, there is also the problem that the first ten years after exposure to a carcinogen seldom provide much evidence of risk. Longer periods, even 30 years or more, may be required before the existence of a risk can be properly assessed, and the inclusion of large numbers of subjects who have been observed for only a short period will serve to dilute the results still further. The analysis should, therefore, be identified whenever possible, both by intensity of exposure and by length of time since exposure began.

The exact nature of past exposure at the workplace is often difficult to determine and, in the case of exposure to metals, may be quite complex. Little information may be available on the previous exposure of individual workers, and comparisons may be practicable only between groups of workers employed in particular occupations or on specific processes. Sometimes, when measurements of the intensity of past exposures are not available, these may have to be estimated from the intensity of current exposure in the same occupation or the length of employment may have to be used as a substitute for measurement on the assumption that exposure has been constant over time. Exceptionally, however, if exposure was particularly heavy at a time when labor turnover was high, duration of employment can be dissociated from intensity of exposure and paradoxical results may appear to be obtained.

A special problem in the study of metals is that mixed exposures to more than one metal and to different valencies or oxidation states of the metal under investigation are frequent, and precise specifications of individual components of the ambient pollution may not be available because of limitations in the analytic method. Changes in the nature of the process and in the consequent exposure patterns may also have occurred over time without being recognized.

Two other considerations which have to be taken into account are the adequacy of the measure of risk and the possible existence of confounding factors. The endpoint that has most commonly been used in studies of metal carcinogenesis is cancer mortality; this is adequate for types of cancer with a high fatality rate (e.g., cancer of the lung) but is a poor measure for assessing the risk of developing tumors with low fatality rates (e.g., cancer of the larynx). Potential confounding factors such as personal habits, socioeconomic status, and other occupational exposure are seldom taken adequately into account due to lack of information. Smoking habits constitute one important confounding factor when the hazard of lung cancer is being considered. Differences in smoking habits are unlikely to be large enough to account for a relative risk of lung cancer more than twice that of the general population, but in the absence of detailed information, it is a matter of judgment as to how far smaller relative

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risks that are not due to chance can be attributed to excess smoking, exposure to the metal, or a combined effect of both.

As with all other epidemiological studies, the most important criterion for the evaluation of those relating to metal carcinogenesis is the consistency of the results between studies (i.e., do they all point in the same direction), within each individual study (i.e., does the risk vary with the degree of exposure), and with the results of laboratory tests concerning carcinogenicity in animals and the absorption, distribution, metabolism and mechanism of action of the metal under investigation. It must, however, be borne in mind that there are many ways in which apparent inconsistency can be produced, and it may be difficult to sort out how far it can be due to differences in the design, method of investigation, or analysis of the studies, or to differences in the exposure of the subjects, or biological variation. Positive results in one set of circumstances are not necessarily contradicted by negative results in another, nor vice versa. When major inconsistencies prevent an unequivocal interpretation of the totality of the data, this should be regarded as constituting urgent grounds for further research.

In the rest of our report we have considered in detail the evidence relating to eight metals (in alphabetical order) and follow this with a brief note on other metals. Specific recommendations concerning individual metals and recommendations concerning the detection of occupational hazards of cancer in general are brought together at the end.

Arsenic

There is conclusive epidemiological evidence of an increased risk of lung cancer in the manufacture of arsenic containing pesticides (*1-3*). The exposure has been mainly to inorganic arsenic compounds. No conclusions can be drawn with regard to the carcinogenicity of trivalent versus pentavalent compounds as both forms have occurred in the circumstances in which individuals have been occupationally exposed. The use of arsenic-containing pesticides, often as arsenates of low solubility, has been associated with lung cancer among vintners in Germany (*4*) and in France (*5*), but the data are not conclusive.

The carcinogenicity of inorganic, mainly trivalent arsenic is also evident from many epidemiological studies of men employed in smelters (*6-11*). As a rule, an increase in lung cancer mortality with increasing dose of arsenic has been observed (*6, 8, 10*). It should be recognized that the smelter environment is very complex, and the interaction between arsenic and other environmental pollutants (SO_2, heavy metals, etc.) as well as with tobacco smoking is poorly understood.

Some studies have indicated an increased mortality from lung cancer in populations living near point emission sources of arsenic to air (*11*), but the role of arsenic cannot be assessed because of lack of measurements of exposure and effects of confounding factors.

Exposure to inorganic arsenic can cause nonmelanotic skin cancer. This has been observed following the ingestion of arsenic in drinking water and in arsenical medications in doses amounting to several grams (*12-15*). The form of arsenic in the drinking water has yet to be determined, but in medication the evidence definitely relates to the inorganic trivalent form (*14*).

Cases of hemangioendothelioma of the liver have been reported following exposure to inorganic arsenic from medicinal preparations (*16-19*), contaminated drinking water (*20*), and wine (*21*). The evidence that arsenic was a causative factor in these cases is thought to be conclusive.

A possible association between arsenic and cancer of other sites, most notably of the lymphatic and hematopoietic systems (*2, 10*), needs further investigation. No epidemiological data are available relating to the carcinogenicity of organic arsenic compounds.

Beryllium

Although numerous experimental studies had indicated that a number of beryllium compounds were carcinogenic in experimental animals by several routes of administration (*22*), the results of epidemiologic study up to the end of 1970 (*23-26*) had not demonstrated any consistent evidence of carcinogenicity in men and women employed in primary beryllium production works, or among subjects enrolled in the U.S. Beryllium Case Registry (BCR). Results of follow-up studies of these populations through the mid-1970's have been recently reported (*27-30*). The study populations somewhat overlap, as three reports concern the workers in two factories (*27-29*), while the fourth concerns the mortality of individuals in the Registry (*30*), many of whom were employed in the same factories. Each study demonstrates a significant excess of lung cancer.

The lung cancer mortality observed in the beryllium exposed workers was excessive compared with the mortality recorded for the U.S. general population (*27*) and that in a second industrial population located in the same geographic region (*29*).

The studies demonstrate an excess lung cancer

risk following short-term but heavy exposure to beryllium (*28*). This same phenomenon was observed for individuals who died from beryllium disease, a clinical endpoint previously demonstrated to be associated with occupational/environmental exposure to beryllium (*31*). Our interpretation of the total evidence is that beryllium is the cause of the excess cancer mortality in these groups of employees.

Cadmium

Occupational exposure to cadmium oxide fumes occurs in the smelting and refining of the metal and in the production of copper-cadmium and brazing alloys. Exposure to cadmium oxide dust occurs in cadmium-nickel battery manufacture and in the production and use of cadmium oxide for plating, chemical and plastics industries. Exposure to cadmium sulfide occurs in pigment manufacture. Lesser exposures to cadmium stearates and other compounds also occur. While cadmium has been used increasingly in industry over the past 50 to 60 years, the total number of workers exposed to the compounds of this metal has not been large. In the earlier years of cadmium usage, occupational exposure was often heavy and prolonged, for neither the fume nor the dust are highly irritant unless exposure is massive. With the recognition of chronic cadmium poisoning, many processes were redesigned in the 1960's with enclosure and the installation of exhaust ventilation. As a result, occupational exposure over the past 10-15 years has been greatly reduced from concentrations measured in milligrams per cubic meter to levels measured in micrograms per cubic meter.

The first intimation that cadmium might be a human carcinogen came from a small study in Britain of men exposed to cadmium oxide dust for a minimum period of one year in cadmium-nickel battery manufacture (*32, 33*), where there were four deaths from carcinoma of the prostate when less than one was expected. Exposure to cadmium oxide dust had been heavy and had given rise to chronic poisoning in the work force. In a similar-sized group engaged for a minimum period of two years on the refining of cadmium in the U.S., also under heavy exposure conditions, four deaths from prostate cancer were again observed, all of which occurred more than 20 years after first exposure (*34*). This number is significantly greater than that expected (0.88) from national mortality statistics. In this cohort there was also a significant excess of lung cancer. However, smoking habits were not determined, and there was concurrent exposure to other compounds, in particular to arsenic. Concentrations of arsenic were low when measured at the time of the survey, but there is no information on arsenic levels in the earlier years. In a Swedish cadmium-nickel battery factory, where again heavy exposure to cadmium oxide and nickel hydroxide dusts had occurred in earlier years giving rise to the first reported cases of chronic cadmium poisoning, all workers with more than five years exposure who developed cancer between 1959 and 1975 (*35*) were identified. No significant excess of prostatic or lung cancer was found. Finally, in a Swedish copper-cadmium alloy plant, a small increase in prostatic cancer mortality, but not reaching a significant level, was found in a group of workers with at least five years heavy exposure to cadmium oxide fume (*35*). In each of these studies the number of workers investigated was small, with, in all, 14 cases of prostatic cancer against 5.4 expected.

The most reasonable interpretation of these results is that exposure to cadmium had contributed to the development of prostatic cancer in the exposed workers.†

Injection site sarcomata and interstitial cell tumors of the testis have followed the injection of several cadmium compounds (*36-43*). Prostatic tumors have not been observed, but it is questionable whether the animals studied provide adequate models (*44*).

Chromium

An increased risk of lung cancer has been established for workers in the primary chromate production industry (*45, 46*). This increased risk has been attributed to exposure to an incompletely defined mixture of chromates. An excess risk of lung cancer has also been observed in workers in the chrome pigment production industry (*47, 48*).

Secondary users of chromates other than the chrome pigment industry and secondary users of chrome pigments must be regarded as possibly being at risk, but the epidemiological evidence (*46, 49-51*) is not conclusive.

Both results from the primary chromate production industry and from chrome pigment production are not inconsistent with the concept that slightly soluble chromates are more carcinogenic than the soluble compounds. Increased risk of lung cancer has been reported in platers exposed to soluble compounds (*52*), but the results in two studies

† One member of the Working Group dissociated himself from this statement on the grounds that he considered the data inadequate at the present time to reach such a conclusion.

(*53-55*) are inconclusive.‡ Animal experiments also support the assumption that the slightly soluble compounds are the more active (*56*), but soluble and slightly soluble compounds are equally potent mutagens *in vitro* (*57, 58*).

There is very little epidemiological evidence relating to workers exposed to trivalent chromium, but such as there is does not suggest an increased risk. A suggestion of an increased risk of lung cancer in ferrochromium production can be attributed to exposure to hexavalent chromium in the process (*50*). The assumption that exposure to trivalent chromium does not constitute an increased risk of cancer is indirectly supported by *in vitro* tests for mutagenicity (*59, 60*) and by the observation that only hexavalent chromium passes the cell membrane (*61*), although the trivalent form may be the active agent at the site of action (*61-65*).

No relation between dose and response can yet be determined for the cancer causing effect of hexavalent chromium in men. This is partly because the active agent in the actual circumstances of exposure is unknown, and partly because only limited and unreliable dose estimates can be obtained for workers who have been found at risk for the last 20-30 years. The importance of the chromium that is found in tobacco for the development of lung cancer is not known, nor is it known whether there are synergistic effects between chromate exposure and smoking. Some published data suggest an increased risk of gastrointestinal cancer (*52, 53, 66-69*), but the results are inconclusive.

Cobalt

Occupational exposure to cobalt occurs in the tungsten carbide hard metal industry, in mining and refining of cobalt, in the production of cobalt alloys, and in the handling of cobalt salts. Pulmonary fibrosis has been reported in workers from the hard metal industry (*70, 71*). Despite these excessive exposures, there have been few reports of cancer mortality occurring in these workers. One epidemiological study of cobalt refinery workers has reported an excess of deaths from lung cancer (*72*). However, interpretation is complicated by the fact that there was also heavy exposure to arsenic, mainly as arsenic trioxide, and probably also to nickel. There are no convincing reports of cancer arising from the use of cobalt-containing prosthetic devices in patients.

Finely divided cobalt metal powder, cobalt oxide, and cobalt sulfide have produced fibrosarcoma (*73*) and rhabdomyosarcoma (*74*) at injection sites in the rat.

Cobalt, as the divalent ion, has been shown to substitute for Mg^{2+} as an activator for DNA polymerase (*75*). Cobalt chloride has decreased the fidelity of DNA synthesis *in vitro* (*75, 76*).

Iron

This most abundant metal in the earth's crust is present in its natural state in the form of oxides and carbonate. Occupational exposure, dating back to prehistoric times, is both common and heavy in iron ore mining, iron refining, steel production and welding.

The injection of certain iron carbohydrate complexes in large doses has been followed by local sarcoma production in several animal species (*77-82*) with evidence of a dose response relationship. Millions of doses of such complexes have been given parenterally over the past 30 years in the treatment of iron deficiency anemia, but no more than a handful of tumors at the site of injection (where tumors are also known to occur spontaneously) have been reported (*83*). In one study of all soft tissue sarcomas which occurred over a defined interval, no history of parenteral iron therapy could be obtained (*84*). In another study covering all cases of sarcoma of the buttock notified to U.K. cancer registries over a two-year period, four cases were discovered (*85*), but only one of them is considered acceptable as a sarcoma that could have arisen in response to iron injection (*86*). The possibility that iron dextran injection may give rise to sarcoma cannot be entirely discounted, but the risk (if any) appears to be extremely small.

The administration of iron oxide to the respiratory tract of hamsters by intratracheal instillation together with benzo(a)pyrene or by inhalation following systemic administration of diethyl nitrosamine has resulted in an increased yield of tumors, while tumors were not obtained with iron oxide alone. This effect is considered to be nonspecific.

An excess mortality from lung cancer has been observed in iron ore miners from a number of countries. The original observations were reported from Cumberland, England (*87*), and confirmed in a later study in 1970, in which the miners of haematite showed a clear excess mortality from lung cancer compared with national and regional figures (*88*). An excess lung cancer mortality has also been reported amongst iron ore miners from some other countries (*89-93*).

The increased lung cancer risk in haematite miners may be attributed to a number of possible factors. Although a carcinogenic effect of iron oxide

‡ Some members of the Working Group felt that the results were conclusive evidence of an increased risk in platers.

particles alone, possibly acting in a nonspecific way, cannot be entirely discounted, the totality of the evidence strongly suggests that the excess lung cancer risk could be attributed to ionizing radiation from the radon and radon daughters present in the atmosphere of the mine. The relationship between the excess mortality from lung cancer and the level of radiation in the Cumberland haematite mines was comparable to that found in other mining situations where radioactivity is held to be responsible for an occupational lung cancer risk (*88*). However, radioactivity levels have not been determined in all other mines where an increased lung cancer risk has been shown to exist.

Two early studies in Britain showed an increased lung cancer mortality in foundry workers (*94, 95*), but this cannot necessarily be attributed to iron oxide, as exposure to polycyclic hydrocarbons also occurred.

Lead

Inorganic lead has been shown to produce renal tumors in a number of experimental studies, at least six in rats (*96-100*) and one in mice (*103*). Dosage was by several routes including oral. Levels of exposure in all of the studies far exceeded the maximum doses tolerated by man and caused gross morphological damage to the kidney. Lead is reported to have produced lung tumors in hamsters (*104*) and cerebral gliomas in rats (*101*). *In vitro*, lead oxide has been found to induce dose-related transformation in Syrian hamster embryo cells (*105*). Lead oxide also enhanced the transformation produced in Syrian hamster cells by SA7 virus (*106*).

In an early epidemiological study, an excess proportionate mortality ratio was noted for malignant neoplasms (all sites combined) in battery workers with so-called "negligible exposure with lead in urine values within the normal range" (25 deaths observed vs. 14.2 expected; $p < 0.05$), but no excess mortality was noted among workers with "heavy" exposure to lead (*107*). No comment was made in that study as to any site-specific occurrence of excess malignancy.

In a more recent study of lead smelter and battery workers (*108, 109*), a significant excess of malignancies at all sites combined was found in smelter workers but not in battery workers. In both groups, mortality from digestive and respiratory cancer was greater than national rates predicted. A five-year follow-up study leaves the interpretation of these findings in doubt. This pattern was not maintained, malignant neoplasms showing a slight deficiency in smelters and slight excess in battery workers (*109, 110*).

From the data it may be concluded that there is evidence that lead in high dose can produce cancer in experimental animals. However, insufficient observations on man have been reported to enable a definite conclusion to be reached as to whether lead causes human cancer.

Nickel

Nickel is used chiefly in the production of alloys including stainless steels. Other uses include: electroplating, catalysts, coinage, and pigments. Nickel alloys are also used in jewelry and in dental and surgical prostheses.

Occupational exposure occurs in mining, refining, alloy production and use, electroplating, welding, and in the handling of nickel salts. Increased risk of lung and nasal sinus cancer in nickel refining workers was first noted in 1926 from Clydach, Wales (*111*), a refinery using the Mond process which began operation in 1902. The Mond process made use of nickel carbonyl in the refining of nickel. Because of this, it has often been believed that nickel carbonyl was the sole carcinogen. Subsequent epidemiological studies from Canada (*112, 113*), Norway (*114, 115*), the U.S.S.R. (*72*), and New Caledonia (*116*) have shown that the increased risk also occurred in refineries where the Mond process was not used. In addition, continuing studies have shown that the increased risk at the Clydach refinery has dropped markedly in workers entering employment after 1932 despite continuing use of the carbonyl process (*111*). Studies in Canada (*102, 113*) and in Wales (*111, 117*) suggest that exposure to furnace fumes and dusts from the high temperature sintering or calcining of impure nickel sulfide mattes was the source of the increased risk. These same operations were carried out in the Norwegian refinery (*118*) and, although some risk appeared to be associated in the electrolytic workers, mixed exposures of these workers cannot be ruled out. The Norwegian studies also demonstrated an increase in the observed incidence of laryngeal cancer, as well as an increase in incidence of lung and nasal sinus cancer.

Mining, smelting, and refining of laterite (nickel oxide) ores have been carried out for about 100 years. It was believed that the increased risk of respiratory cancer did not occur in laterite operations. A single case-control study, however, reported an increase among workers and residents living near a nickel smelter in New Caledonia (*116*). It was also assumed that increased risk of respiratory cancer did not occur among nickel users, e.g., in nickel alloy manufacture and use, use of nickel powder, nickel plating, nickel welding, etc., but

definitive studies on this have been lacking. A recent study did not indicate an increase in respiratory cancer among a cohort exposed to nickel powder and followed for a minimum of 19 years (*119*). Another recent case control study did not indicate an increase of risk in workers engaged in grinding and welding operations with nickel alloys (*120*).

Studies with experimental animals have demonstrated that nickel subsulfide (Ni_3S_2) produced local tumors at a wide variety of injection sites and by inhalation in rats (*121*). Ni_3S_2 also enhanced the carcinogenic effects of benzo(a)pyrene (*122*) and 20-methylcholanthrene in experimental animals (*123*). *In vitro* mammalian cell tests demonstrate that Ni_3S_2 and $NiSO_4$ compounds give rise to mammalian cell transformation (*124-130*).

Other Metals

Epidemiological evidence relating to exposure to a variety of other metals should be sought if at all possible, either because large numbers of workers are involved or because laboratory tests suggest the possibility that a cancer hazard might exist. These metals include copper, manganese, mercury, platinum, selenium, titanium, vanadium, and zinc.

Studies of copper refiners are particularly desirable, but it may be difficult to define groups who have not also had material exposure to arsenic.

In view of the small numbers of workers exposed, it will be necessary to obtain the collaboration of industry on an international scene if any worthwhile human evidence is to be obtained relating to platinum, selenium, and vanadium. The possibility of establishing specialized registers of men who have worked with these metals should be considered.

Recommendations

Specific

The epidemiological evidence strongly implicates inorganic trivalent arsenic as the responsible agent when an excess of lung cancer is attributed to exposure to arsenic. There is, however, no reason to exonerate pentavalent arsenic, and workers exposed solely to other forms of arsenic should be investigated further.

Experimental work should be undertaken on the effect of exposure of the respiratory tract to arsenic compounds of low solubility.

The incidence of lung cancer in areas where there are unusually high concentrations of arsenic in the air should be studied in order to assess possible risks to the general public. Airborne levels and personal excretion patterns should be monitored in such areas.

There is a need to study dose-response relationships for arsenic in water and skin cancer. Particular emphasis should be given to differences in relation to valence state.

The conclusion that beryllium and cadmium contribute to the development of some cancers in man was reached because this seemed to be the most reasonable interpretation of the available facts. The numbers of observations on man are small, however, in both cases, and it is important to check that the conclusion is correct by continued observation of the cohorts of workers exposed to beryllium in the U.S. and by collecting observations on larger numbers of men who have been exposed to substantial amounts of cadmium whenever they can be found.*

Further studies are required to determine which compounds of chromium are carcinogenic to man and, particularly, to determine if there is any hazard associated with exposure to soluble compounds in the plating industry.

Cohorts of workers exposed to airborne respirable cobalt and its inorganic compounds should be identified and followed up to determine their cancer mortality rates.

Long-term exposure of experimental animals to both insoluble and soluble forms of cobalt (such as cobalt oxide or cobalt sulfide and cobalt chloride or cobalt nitrate) via the respiratory tract should be included.

Further epidemiological studies of iron miners, steel workers, foundry workers, and welders are required in which attempts are made to characterize the extent to which the men are exposed to ionizing radiation and possible carcinogens as well as to iron and iron compounds.

More studies are needed of men who have had long term occupational exposures to lead. Case-control studies of adults with kidney tumors and follow-up studies of people who suffered from lead poisoning in childhood could also help to clarify the extent to which lead may contribute to the development of cancer in man.

Further epidemiological studies of workers exposed to soluble and slightly soluble forms of nickel and nickel alloys in the nickel processing and mining industries are needed.† These should in-

* A larger epidemiological study of cadmium workers is currently being carried out in Britain, the results of which should be available in 1982.

†Many such studies are currently underway, the results of which should become available in 1981.

clude industries involving mixed exposures to nickel and other metals, e.g., nickel and cadmium in battery manufactures and nickel and chromium in stainless steel manufactures, welding, nickel plating, and chrome plating.

Additional long-term inhalation studies in experimental animals are needed to determine the potential carcinogenicity of nickel metal and nickel compounds. Priority should be given to testing nickel metal, nickel sulfate, and nickel oxide, because of the large number of workers exposed and the lack of conclusive information concerning these substances.

General

The possibility of interactions between metals (particularly arsenic), other industrial pollutants and tobacco smoke in the production of lung cancer should be further investigated.

Epidemiological studies should, whenever practical, include comparison of the risk of cancer in groups of industrial workers exposed to a particular agent with that in unexposed workers in the same industry or in residence in the same geographical area as well as with those in the country as a whole.

In epidemiological studies, identification of cohorts with past exposure is often difficult or impossible because: (a) employment records are often destroyed after a minimal time interval, and (b) job descriptions in company or medical records may be nonexistent or inadequate. Employment records adequate for the identification of workers for the determination of their vital studies should be retained by companies and medical or industrial hygiene departments where these exist for a minimum of 30 years. Because of the long period that may elapse between initial exposure and the development of cancer, these records should include job description, with qualitative and quantitative information on exposure. Whenever possible, information on other potential exposures, e.g., cigarette smoking, should be recorded.

In the interest of public health, facilities should be provided in all industrialized countries to allow bonafide research workers to link records maintained in industry with national records of birth (when practicable), sickness, and causes of death, subject to appropriate safeguards to insure that personal records are never used to the detriment of the individual.

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Experimental Studies in Whole Animal Bioassay*

Animal models have been used for a number of purposes in metal carcinogenesis studies: (a) to detect carcinogenic activity; (b) to estimate carcinogenic risk; and (c) to investigate mechanisms of metal carcinogenesis.

The design of animal tests will be determined by the purpose of the study. In this section we shall distinguish between tests in animals designed to identify carcinogenic activity versus tests intended to have special relevance to the evaluation of effects in man.

Tests of Carcinogenic Activity

General Principles

Guidelines for decisions relative to the carcinogenicity of a metal in experimental animals will include the determination of the purity, the speciation, and the physical state of the metal compound being evaluated in the experimental animals (*1*).

At the present state of knowledge, short-term or *in vitro* tests are valuable research tools for metal carcinogenesis studies, e.g., molecular mechanisms, but no test or battery of tests has been sufficiently validated to be used reliably in predicting the carcinogenic activity of metal compounds in animals. Tests in whole animals are necessary for the experimental identification of carcinogenic activity.

The intramuscular route of administration has been used to study the carcinogenic potential of numerous metal compounds. Tumors appear at the site between 4 to 11 months (a few first appear after 12 months). The subcutaneous route is the second most frequent one by which positive results were obtained with metals. Solid-state carcinogenesis may complicate the interpretation of tumors which appear at the site of implantation by this route. Occurrence of tumors only at the site of injection should be followed by studies employing other routes of administration.

Intratracheal instillation has been employed to study the carcinogenic or enhancing effects of several metal compounds. Larger doses can be administered, and maximum tolerated dose can be approximated by this route of administration.

The oral route has been traditionally used for carcinogenic studies of nonmetals. This route has limitations for metal studies since some metals are poorly absorbed from the gastrointestinal tract. Diet and water must be analyzed for metal content. Most commercial rodent diets are high in calcium, zinc phosphates, and phytates, which interfere with metal absorption. The metal being studied must also be analyzed in the control diet. Blood and urine levels of the agent under test must be measured to obtain some indication of the amount of the metal absorbed.

Special routes of administration may be used to answer specific questions, such as the intrathoracic route to induce mesotheliomas.

It must be recognized that, although a metal may be carcinogenic in one or more species or strains, e.g., in rats, mice, hamsters, no generalization can be made about interspecies comparison. Species differences in relation to metal metabolism can have profound effects on the outcome of experiments. From a practical viewpoint, rodents are the animals of choice, but more research is needed to determine the carcinogenic response of other orders. Bioassays on more than one species are

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necessary to reduce the possibility of missing the carcinogenic action of a metal.

A major area of research which has been neglected is transplacental and perinatal carcinogenesis by metals. Here the experiments must be so designed as to differentiate intrauterine exposure, neonatal exposure, and exposure through the maternal milk.

Relation to *in Vitro* Studies

Presently, short-term *in vitro* bioassays are under development for detection of metals as carcinogenic agents via both genetic and nongenetic pathways. Current research efforts directed toward improvement of *in vitro* techniques for the detection of metal carcinogens continue to rely heavily on information obtained from *in vivo* bioassay. It is expected that, with further development of *in vitro* bioassays, complex mixtures and key elemental interactions may be screened *in vitro* prior to *in vivo* testing. Furthermore, because of the flexibility of *in vitro* bioassays, such systems may provide clues on how to conduct *in vivo* tests in the most efficient manner. A major difference, however, between *in vitro* systems and the *in vivo* assays relates to chemical speciation. *In vivo* response is markedly dependent on chemical form, i.e., Ni_3S_2 vs. NiS vs. $NiSO_4$, and low and high temperature forms of BeO. Understanding of the effects of different chemical species of metals, however, will provide insight into the mechanism of metal carcinogenesis.

Estimation of Carcinogenic Risk

Comparisons of Carcinogenic Activity

Carcinogenic activity in animal experimentation relates to the dose that causes a given frequency and incidence of cancer under controlled experimental circumstances, species, strain, age, and sex of the experimental animals. The ability to detect carcinogenic activity is also determined by number of animals, positive and negative controls, period of observation, and so forth. Carcinogenic activity is a relative term and implies a comparison with other chemicals such as weak versus strong carcinogens (*2*).

Measurements of carcinogenic activity are used for experimental purposes, for example, in studies of structure-activity relationships, identifying the "proximate" carcinogen, or assessing the effects of modifying factors on the carcinogenic response. Measurement of weak versus strong carcinogenic substances in experimental animals have been extrapolated as estimated of relative risks in the human populations for exposures to one substance versus another.

Relevance of Animal Tests to Man

In order to predict hazard to man from animal studies, there must be judicious selection of test species, routes of exposure, and dose. Tests such as *in vitro* tests and/or *in vivo* tests such as intramuscular or subcutaneous injection may or may not precede the use of more relevant exposure techniques.

Selection of Test Animals. Because of ease of handling, accumulated long-term experience, and the capability of the investigator to manipulate fairly large groups of animals, it is likely that the animals chosen for whole animal tests will, in most instances, be standard laboratory rodents, that is, mice, rats, and hamsters. It is essential, however, to recognize the possible shortcomings of this approach. Absorption, transport, storage, excretion, and qualitative and quantitative aspects of biotransformation of metal compounds may vary considerably among rodents and between rodents and humans. Similarly, differences in interaction with target macromolecules and differences in repair capability may be significant determinations of variations in response.

The use of two or more test species will reduce the likelihood of confusing species-specific responses from those applicable to man. Certainly, differences in response among rodent species should be exploited to illuminate the mechanisms of carcinogenesis.

Routes of Administration. An experimental protocol designed to assist in the estimation of human risk should preferentially employ routes of administration identical to or as similar as possible to those involved in human exposure. The route chosen will determine the effect of the test material on tissues immediately contacted as well as on tissues ultimately reached by absorbed and metabolically altered products. Thus, feeding experiments alone are not sufficient to examine systemic carcinogenicity of a material poorly absorbed through the intestine as is the case with many metal compounds. Some metals, although poorly absorbed, may nevertheless accumulate over a lifetime.

Dose. It has become customary to utilize the estimated "maximum tolerated dose" in the design of initial bioassays in animals. The justification for this choice has been the necessity to explore the full

range of effects of a test agent and also the limitation of detectable effects imposed by use of a relatively small group of animals. These should be considered preliminary studies to demonstrate the carcinogenic potential of the substance. However, extrapolation of findings to long-term, lower level exposure in larger groups of humans may require studies at different dose levels. For example, high dose metabolism may differ significantly from metabolism of lesser quantities of metal compounds. High doses, not inconsistent with those occasionally encountered by humans, may produce severe interfering toxic effects. Accordingly, the experimental design should include lower doses that do not produce such toxicity. Patterns of dosage may include such things as intermittent exposure, short-term high level exposure followed by prolonged observation, or even single dose exposure, particularly to refractory materials with long biological half lives.

Careful attention must be given to those determinants of dose other than total quantity. These include such factors as particle size, solubility, stability, and the metabolic pathways which determine ultimate dose to the target tissue and cell.

Comparison of Animal Studies with Epidemiological Findings in Man. Bioassay of metal compounds in experimental animals may be predictive of effects in man. Although there are a number of quantitative differences between various species of laboratory animals and man, there is a basic universality of biological systems that permits extrapolation of effects in animals to man (*3-5*). Ideally, animal studies should provide sufficient information regarding the carcinogenic potential of metal compounds to support regulatory measures that are protective to human health. In those instances where human effects have already occurred or are suspected, animal studies may confirm the activity of a specific substance and route of exposure and may also elucidate mechanisms and factors that influence effects in man.

To date, exposures to arsenic, beryllium, cadmium, chromium, and nickel (or their compounds) have been identified as contributing to the development of human cancer (*6*). Certain compounds of beryllium, nickel, and chromium, which are accepted as contributing to pulmonary cancer in man, also produce tumors in animals exposed via the respiratory tract. Animal models treated by similar routes of exposure produce tumors with compounds of these metals.

Arsenic has been linked through epidemiologic studies to skin cancers following ingestion of As in drinking water and Fowler's solution and to carcinoma of the lung in workers exposed by inhalation (*7*). In spite of extensive investigation in diverse animal species, there is no definite evidence that arsenic is carcinogenic for experimental animals. Recent studies, however, involving intratracheal administration of a mixture containing arsenic, copper sulfate, and calcium oxide resulted in pulmonary tumors (*8*). These findings point to differences of susceptibility to arsenic compounds between man and certain experimental animals. Also, the epidemiologic studies may reflect other mixed exposures of which arsenic is only one component of exposure to specific forms of inorganic arsenic. Prostatic cancer has not been found following administration of cadmium by gastric gavage or injection, but these studies resulted in low tissue content of metal (*9*). There have been no reported long-term studies of inhalation of cadmium compounds in animal models for carcinogenesis. Intramuscular and subcutaneous injection resulted in sarcomas at the site of injection. Testicular tumors were found after systemic intratesticular injection in fowl.

A number of metal compounds have been reported to cause tumors in experimental animals, but corresponding carcinogenicity has not been established by studies in man. Cobalt, manganese, titanium, zinc, and carbohydrate-iron compounds each produce tumors by parenteral routes at the site of injection, a route of exposure not likely to occur in man (*10*). Such studies by injection routes may, however, provide incentive for further studies employing other routes of exposures. Also, such studies may be predictive of implant effects, perhaps relative to usage of materials for prosthesis, although the role of physical factors as well as the chemical nature of the metal compound must be considered in such evaluations.

Lead is the only metal to date that produced tumors in rats following oral administration (*11*). There are some epidemiological data regarding the carcinogenicity of lead in man (see Epidemiology Report). These data, however, do not provide sufficient evidence to support definite conclusions as to whether lead causes human cancer (*12*).

Differences in susceptibility to toxicity between man and experimental animals may permit levels of exposure to the metal in experimental animals that cannot be tolerated in man, thus leading to discrepancies between epidemiological observations and animal studies.

It is concluded that the relevance of animal studies to man is closer when the same metal compound produces tumors by similar routes of exposure in man and animal models, produces tumors in common target organs, and produces tumors in different animal orders.

Mechanisms of Metal Carcinogenesis

Route of exposure appears to be an important factor in metal carcinogenesis. It is generally difficult to produce tumors by adding metals to food or water, since absorption from the gastrointestinal tract is often poor.

Metabolism is an important aspect in considering mechanisms of metal carcinogenesis. Quantification of initial deposition of the metal in target tissues (lung, skin), eventual translocation to susceptible tissues (bone, kidney), and persistence of metals within their target are important elements in understanding and possibly predicting mechanisms.

A substantial amount of data indicates that solubility, valence state, oxidation-reduction reactions, and formation of complexes are important determinants in metal carcinogenesis (*13*). It will not be adequate to obtain *in vivo* data on the behavior of metallic cations only. Experimental approaches need to be developed which allow the study of possible biotransformation and behavior of metals in complex biological surroundings.

It is the prevailing hypothesis that some metals cause cancer by inducing damage directly to the genome (*14-18*). The evidence has been summarized in recent reviews (*1, 19*). Less data from whole animal studies are available to support the hypothesis. Such information should become available. However, it would appear important that the experimental model be carefully selected. Unless a given experimental approach will produce a high tumor response *in vivo*, it will not be possible to link, with confidence, molecular or cellular changes directly to the eventual development of cancer.

Metal compounds may also produce tumors through nongenetic mechanisms. One notion is that metals are promoters. Furthermore, their effects on intracellular metabolism make cells more susceptible to initiation. Certain platinum compounds have been shown to be carcinogenic in animals and to act as initiators for the mouse skin (*20*). There is a large amount of information available on how metals inhibit enzymes, bind to cellular macromolecules other than DNA (nuclear proteins, RNA), bind to receptors or interfere with membrane functions. Some of these adverse effects are quite specific for either a particular enzyme or for a particular metal. They often occur at metal concentrations which are orders of magnitudes below those found to induce genetic damage. In at least some circumstances, metal carcinogenesis may be mediated by trophic hormone effects. A possible example is induction of Leydig cell tumors in rats following testicular necrosis after subcutaneous injection of $CdCl_2$ (*21*).

The relation of immune response to metal carcinogenesis has received too little attention thus far. Certain metals (e.g., Pb) appear to suppress the general immunocompetence of the host, but other metals (e.g., Be) were shown to have allergenic properties. With beryllium, inhalation exposure has suppressed a previously induced cutaneous hypersensitivity to the same ionic species of the metal (*22*).

Metal interactions in carcinogenesis have recently been reviewed (*23-25*). Interactions may be divided into the following areas: (a) metal antagonism or synergism with metal carcinogens; (b) metal antagonism or synergism with organic chemical carcinogens; and (c) biological factors affecting the expression of chemical carcinogenesis. A major limitation in interpreting the health significance of co-exposure from complex mistures is the lack of specific information on the chemical form of the biologically active element.

Clearly, from a toxicological and nutritional standpoint, metal-metal interactions are well documented, i.e., Zn-Cd, Ca-Zn, Cu-Zn, Se-Hg, Se-Cd, etc. Few carcinogenesis studies, however, have examined such interactions. Co-exposure of zinc acetate and cadmium chloride (*21*) or manganese metal and nickel subsulfide (*26*) have resulted in substantial reductions in tumor incidence. Metabolic studies of the Mn-Ni system have not elucidated the mechanism of antagonism. Thus, further work is required to evaluate the carcinogenicity of co-exposures. Furthermore, in light of the increasing evidence of varied essential and nonessential elemental exposure in human populations, nutritional status must be considered in the evaluation of the totality of human exposures to trace elements.

Trace element exposure often occurs in combination with organic chemical carcinogens. Inhibition of carcinogenesis has been described with copper and selenium compounds. Azo-dye hepatocarcinogenesis may be entirely suppressed by dietary copper supplementation probably due to more rapid catabolism via azo reduction (*27*). On the other hand, copper protection against ethionine induced hepatomas is thought to be due to decreased catabolism and increased hepatotoxicity (*28*). Such studies demonstrates the potential complexity of metal interactions.

The best evidence of synergism results from studies of respiratory tract co-exposure to inorganic particles and organic carcinogens (*29*). Although the mechanism is not completely understood, rate of release of carcinogen and particle size appear to be important factors in the incidence of respiratory tract tumors. Arsenic activity might be related to exposures of mixtures. However, the

role of arsenic as a cocarcinogen is unclear. Recent studies indicate an important role for trace elements as regulators of the immune system as well as activators of viral expression. For example, dietary zinc status may alter the growth rate and incidence of metastases of transplanted tumors (*30*).

Recommendations

1. The investigation of metabolic conversions involving oxidation states *in vivo* and *in vitro* and the relationship to biological effects should be studied.
2. The development and improvement of analytical methods should be undertaken to identify biologically active chemical species.
3. The investigation of the role of immune response to metal compounds and metal hypersensitivity for metal carcinogenesis should be examined.
4. Investigation of the role of metals as cocarcinogens and promoters need to be investigated. Animal as well as *in vitro* models are required for examination of possible promoting activity for metal compounds.
5. Analytical techniques for speciation of metal compounds in complex mixtures (e.g., coal fly ash) and in body tissues should be developed.
6. There should be consideration of more research on the carcinogenic response of animals other than rodents, such as avian and aquatic types (*10*, *11*).
7. Further studies of carcinogenesis resulting from perinatal and transplacental exposure are required.
8. The synergism and antagonism between metal compounds, particles and organic chemical carcinogens, and the underlying mechanisms, should be investigated.
9. Methods relevant to the carcinogenesis testing of alloys and metallic substances that are being used for prostheses for dentistry, orthopedics and for artificial internal organs should be developed.
10. The role of chemical and physical forms in their carcinogenicity should be investigated for selected metals.
11. The assessment of carcinogenic activity in an animal model which suggests the possibility that a metal compound may have a cancer inhibitive or an anticarcinogenic effect should be carefully defined and given emphasis in the planning of further investigations. The possibility that selenium and zinc in certain circumstances may have anticarcinogenic effects requires further investigation.

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